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DRUG DEVELOPMENT RESEARCH 33:174–188 (1994)

Research Overview

Recent Advances in the Generation of Chemical Diversity Libraries

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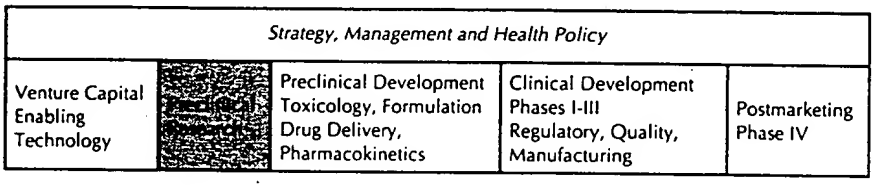

ABSTRACT In recent years, screening in combination with a diverse compound collection has become a powerful method for discovering leads for the ever-increasing number of new biologically active peptides, proteins, receptors, and enzymes discovered continually. As a result, the rapid generation and screening of compound libraries (collections) have recently become important major tools in the search for novel lead structures. Diverse collections of compounds have been acquired by many strategies; these include (1) natural products from plants, fermentation, marine organisms, insect toxins, and ethnic pharmacotherapies; (2) recombinant randomized peptide libraries (often referred to as biological diversity); (3) multiple peptide synthesis; and (4) non-peptidic synthetic libraries. The present review provides an overview of the recent advances in the field of peptide and non-peptidic synthetic libraries. The progress made thus far is broadly divided into two categories: (1) Amide based libraries. These libraries share the concepts of the peptide library strategies; much of the referenced work thus refers to peptides, reflecting the bias of the literature to date. (2) Non-amide based libraries. This promising technology combines solid phase synthesis with classical organic synthesis to provide large numbers of compounds with desirable bioavailability and pharmacokinetics for screening. The basic premise behind the second approach is that the high affinity ligands, when identified, will be much more likely to become useful therapeutic agents than the compounds discovered from amide based libraries. Synthesizing small heterocyclic ring systems that use ligands of diverse biological activity via combinatorial strategies is a fast developing branch of medicinal chemistry. We are at an early state in the development of combinatorial chemistry. However, this dramatic convergence of technologies represents a fundamental advance in medicinal chemistry and promises to play a major role in future drug discovery efforts. © 1994 Wiley-Liss, Inc.

Key Words: molecular diversity, solid-phase synthesis, peptoid, combinatorial chemistry

INTRODUCTION

Recent advances in molecular biology, pharmacology, and automation have allowed the testing of minute amounts of compound ($\leq$ 1 nanomoles) in a relatively short time in a receptor binding or an enzymatic assay of interest. To the major drug companies who have collected large numbers of compounds (usually $\geq$ 100,000) over the years via chemical laboratory synthesis, there has existed a competitive edge in utilizing them for the discovery of new lead structures via screening. Many of the initial leads of recently reported bioactive compounds have been dis-

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covered by random screening of these corporate compound inventories [Clozel et al., 1993; Hsu et al., 1991; Snider et al., 1991]. Thus, screening in combination with a diverse compound collection can be a powerful method for discovering leads for the ever-increasing number of new biologically active peptides, proteins, receptors, and enzymes discovered continually. In contrast, the biotech companies without any compound collections, find themselves at a competitive disadvantage in the discovery of leads via screening. Thus, it is no surprise that companies such as Affymax, Chiron, Houghten, and Selectide, to name a few, have taken the lead in the area of generating chemical diversity in the form of combinatorial libraries. As a result, the rapid generation and screening of compound libraries have recently become important major tools in the search for novel lead structures.

Diverse collections of compounds have been acquired by many strategies; these include (1) natural products from plants, fermentation, marine organisms, insect toxins, and ethnic pharmacotherapies [Hylands and Nisbet, 1991]; (2) recombinant randomized peptide libraries (often referred to as biological diversity) [Bull et al., 1992]; (3) multiple peptide synthesis [Jung and Beck-Sickinger, 1992] and (4) nonpeptidic synthetic libraries [Pavia et al., 1993; Gallop et al., 1994; Gordon et al., 1994]. The range of diversity has extended in a major way beyond natural products only in the last decade. Progressive chemical and biological means for artificially generating diversity have now far outstripped the libraries of the past, with millions upon millions of compounds readily available through a variety of approaches. Recombinant systems such as bacteriophage excell in producing hundreds of millions of peptides from a block set of 20 standard amino acids (19 L-amino acids plus glycine); in contrast, the chemical synthesis strategies may be less persuasive in terms of numbers, but their diversity may be limitless because of the endless variety of building blocks possible in such an approach.

In compound libraries the compounds are synthesized and stored as mixtures; sometimes the compounds, however, are synthesized individually in pools. This feature permits the synthesis of large numbers of compounds in a relatively short time; also the screening time is considerably reduced. For these libraries to be useful for discovering lead structures, there are subtle issues that must be balanced against each other. The faster pace of synthesis is achieved by generating deliberate mixture of compounds per vial. Logically, the library can be synthesized at a faster pace by increasing the number of compounds per vial. However, this must be weighed against the time it takes to deconvolute a mixture. Toward this objective ingenious detection protocols have been formulated [Chu et al., 1992; Kerr et al., 1993; Nikolaiev et al., 1993; Ohlmeyer et al., 1993]. The magnitude of mixing order also affects the individual concentration of components in a bio-assay; thus it has a direct effect on the activity of the lead structure that will be discovered. As one would expect, the higher the mixing order, the more potent will be the lead structure and statistically fewer will be disocvered that bind to the target. Further complicating the matter is the identification of reliable chemistry that would allow the synthesis of medicinally interesting structures in reasonable amounts and rapid time from readily available starting materials. Other issues that have an impact on library strategy include automation of the process, the data handling capacity, the availability of key building blocks, the quantity of each compound synthesized, and the molecular weights of the oligomers, which may determine ultimate absorption, distribution, metabolism and excretion parameters. *Not surprisingly, a fruitful library strategy requires carefully balancing various opposing issues such that the probability of finding a lead structure for a given assay with desirable bioavailability and pharmacokinetics parameters is increased.*

Almost all library protocols described to date rely on solid-phase synthesis techniques. An advantage offered over solution based methods is that the reactions can be driven to completion by employing excess reagents; product isolation is accomplished simply by washing away excess reagents from support-bound material. In recent years, a variety of polymers have been described for synthesis; these include cellulose discs [Eichler et al., 1989a,b; Frank, 1992a,b, 1993; Frank and Doring, 1988], cellulose paper sheets [Gausepohl et al., 1992], cotton fabric [Eichler et al., 1991], Kel-F-gstyrene and polystyrene-1% divinylbenzene [Albericio et al., 1989], long-chain polystyrene-grafted polyethylene film [Berg et al., 1990a,b], and controlled-pore glass resin [Chong et al., 1992]. Nonetheless, the resin obtained from polymerization of polystyrene-1% divinylbenzene continues to be the support of choice.

In the last decade, Geysen et al. [1984], Houghten [1985], Lam et al. [1991], and others using variants of the solid phase peptide synthesis (SPPS) independently generated libraries having treater than tens of thousands of peptides. The combinatorial relationship between the length of an oligomer and the number of variants at each position made the synthesis of large libraries possible. An oligomer of length n with X variants at each position will theoretically give X^n compounds (Table 1).

TABLE 1. Relation Between X^n and Number of Distinct Molecules

Number of amino acid residues (X^n)	Number of peptides
2	400
3	8,000
4	160,000
5	3,200,000
6	64,000,000
7	1,280,000,000
8	25,600,000,000

With solid phase peptide synthesis, pioneered by Merrifield [1963] as the state of the art, libraries of peptides were synthesized and used in drug research. The drawback is that, unlike compound inventories at major drug companies, these libraries contain only biopolymers from natural amino acids, their enantiomers, or a small number of unnatural amino acids. The metabolic instability of these compounds, owing to proteolysis, and their poor absorption characteristics, often render them poor drug candidates.

The present review provides an overview of recent advances in the field leading to non-peptidic synthetic libraries. The progress made thus far is broadly divided into two categories: (1) Amide based libraries. These libraries share the concepts of peptide library strategies; much of the referenced work thus far refers to peptides, reflecting the bias of the literature to date. (2) Non-amide libraries. This promising technology combines solid phase synthesis with classical organic synthesis to provide large numbers of compounds with desirable bioavailability and pharmacokinetics for screening. The bias behind the second approach is that the high affinity ligands, when identified, may be more likely to become useful therapeutic agents than the compounds discovered from amide based libraries (whether this proves true or not remains to be seen). Table 2 lists the key contributors in the field of combinatorial chemistry.

TABLE 2. Representative Companies Involved in Generating Combinatorial Libraries

Libraries	Representative companies
Carbamates	Affymax, Chiron
Carbohydrates	Glycomed, Ontogen
Natural products	Shaman, Xenova, MYCOSearch
Non-peptide amides (peptoids)	ArQule, Chiron, Houghten, Pharmacopeia, Selectide, Sphinx-Genesis
Oligonucleotides (biological)	Darwin, NeXagen, Evotech
Oligonucleotides (chemical)	Darwin, Gilead, ISIS
Peptides (biological)	Affymax, Chiron, Genentech, Ixsys, Optein, Protein Engineering
Peptides (chemical)	Affymax, Bristol-Myers Squibb, Chiron, Houghten, Lilly, Pharmacopeia, Selectide, Terrapin
Polyamines	Houghten
Scaffold-based	Chiron, Ontogen, Selective
Small molecules	ArQule, Chiron, Parke-Davis, Pharmacopeia
Spatially directed	Affymax, Chiron
Tagged libraries	Affymax, Chiron, Pharmacopeia

AMIDE BASED LIBRARIES

Synthesis of Peptides: "Multi-Pin" Method

In the early 1980s, the field of chemically generated molecular diversity was born when Geysen demonstrated that peptides could be synthesized in a reusable form in numbers that are orders of magnitude greater than ever before [Geysen, 1987, 1991; Geysen et al., 1984].

In this method, peptides can be synthesized on polyacrylate-grafted polyethylene rods arranged in a standard 96-well microtiter plate format. The format allows 96 individual peptides (or mixtures) to be synthesized simultaneously at the ends of the rods (Fig. 1). The technique utilizes conventional SPPS methods, readily available materials and instrumentation, and has been used, for example, to synthesize more than 3,000 peptides in a week. Moreover, peptides can be used in assays while remaining bound to the rods, allowing their re-use for 20 or more tests, or the peptides can be cleaved and then tested in solution-phase assays [Bray et al., 1990, 1991b; Geysen, 1990].

One cleavage method yields products that contain a diketopiperazine moiety at the C-terminus, thereby introducing novel structural diversity upon cleavage (Fig. 2) [Valerio et al., 1991]. Through the use of new linkers or handles for attachment to the solid support, β-alanine acid or β-alanine methyl amide C-termini can also be generated [Valerio et al., 1991]. Alternatively, support-bound peptides can be cleaved with ammonia vapor to afford the corresponding C-terminal primary amides [Bray et al., 1991a]. An amino acid indexer that facilitates multi-pin use has been described [Carter et al., 1992].

The multi-pin approach is ideally suited for preparing a complete set of all possible overlapping synthetic peptides of given length homologous to a peptide sequence of interest [Geysen et al., 1985]. The power of the multi-pin method in pharmaceutical research has been demonstrated recently in replacement net approaches to the determination of structure-activity relationships (SARs) in a series of potent hexapeptides that bind endothelin receptors [Spellmeyer et al., 1993a,b], and in studies of substance P

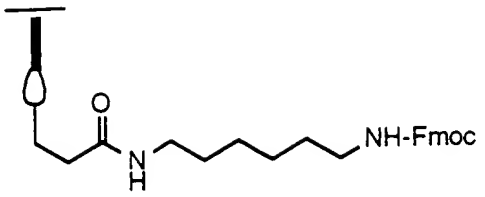

Figure 1. Schematic representation of a pin, derivatized for solid phase synthesis.

[Wang et al., 1993a,b]. A general discussion of systematic screening for bioactive peptides has been published [Maeji et al., 1991].

A recent discussion considers practical strategies for the synthesis and testing of large libraries, taking into account the number of monomers available, as well as various optimization methods [Geysen and Mason, 1993]. The combination of epitope mapping with other SAR methods, made possible by multi-pin or alternative multiple peptide synthesis methods, has been used to enhance tumor necrosis factor (TNF) activity [Rathjen and Aston, 1993]. It has also been suggested that peptide ligands, especially as selected from a vast library of random peptide sequences, may have potential in generating ligands for large-scale affinity chromatography [Baumbach and Hammond, 1992].

Synthesis of Peptides: "Tea-Bag Approach"

Among the first alternatives to multi-pin peptide synthesis was the tea-bag approach [Houghten, 1985], where small amounts of resins representing individual peptides are enclosed in porous polypropylene containers, which resemble tea bags (Fig. 3). Resin-containing tea bags are immersed in individual solutions of amino acids, and standard SPPS procedures are utilized. Deprotection and washing steps are accomplished by mixing the tea bags together in a reaction vessel, followed by their subsequent separation for the next coupling. Finally, peptides are cleaved in a multi-vessel HF apparatus [Houghten, 1986; Houghten et al., 1986]. This method affords significant amounts of fully characterizable, solution-phase peptides, which may yield more relevant data than solid-support bound peptides. Neither analysis nor separation is required for the identification of biologically active peptides, since the biological assay identifies the tea bags that contain active peptides.

A semi-automated process allows the simultaneous synthesis of up to 120 different peptides [Beck-Sickinger et al., 1991]. This method has been extended to a combinatorial approach [Houghten et al., 1991], which permits the rapid synthesis and evaluation of millions of individual, unmodified, free peptides, in quantities useful for screening in solution. For example, from libraries theoretically containing more than one million oligopeptides, nanomolar potency opioid ligands were identified using radioreceptor assays, and the antigenic determinant of a peptide recognized by a monoclonal antibody (MAb 125-10F3) was determined using a competitive enzyme-linked immunosorbent assay (ELISA) [Houghten et al., 1992; Houghten and Dooley, 1993].

Synthesis of Peptoids: "Mixed Resin Method"

As a chemical alternative to natural biopolymers that would be pharmaceutically relevant, Simon et al. have developed "peptoids" [Simon et al., 1992, 1994]. Peptides can be thought of as polymers of glycine with side chains projecting from the α carbon atoms; in N-substituted glycine (NSG) peptoids, the side chains are attached to the amide nitrogen. In these NSG peptoids the achiral, flexible backbone is equipped with functional groups spaced at intervals comparable to those in naturally occurring peptides (Fig. 4).

This provides a structurally flexible molecule with functionalities required for molecular recognition. NSG peptoids are synthesized via a "sub-monomer route" [Zuckermann and Banville, 1992; Zuckermann et al., 1992a,b,c,d]; the sub-monomer route avoids tedious synthesis and deprotection of N-terminal Fmoc-protected glycine building blocks. The construction of NSG peptoids proceeds from the C-terminal to the N-terminal end, with two repetitious steps; firstly the coupling of α-haloacetic acid and secondly the displacement of halide with amine (Fig. 5).

The advantage of these new structures over peptides are many: (1) one would expect them to have improved metabolic stability to natural proteases because of N-substitution, which they do (unpublished data); (2) a greater diversity of side chain functionalities can be incorporated from the large number of commercially available and functionally diverse primary amines; (3) the achiral nature of the backbone simplifies synthetic problems, especially racemization; (4) a higher inherent flexibility provides the possibility of probing a larger conformational space; and (5) the synthetic protocol retains advantageous features of solid phase chemistry, namely, modular stepwise assembly from readily accessible monomers, as well as the capacity for automation [Zuckermann and Banville, 1992]. NSG peptoids are often synthesized via a mix and split method pioneered for peptide libraries, as a mixture of ~500 peptoids per pool. If biological activity is observed, a pool can be deconvoluted via a small number of synthetic cycles to individual active compounds [Simon et al., 1991].

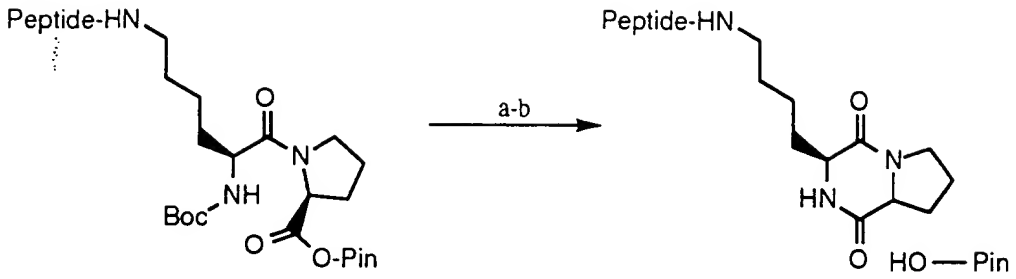

Figure 2. Formation of diketopiperazine linked peptides, which allows screening in solution. Reagents: (a) CF_3COOH; (b) base or buffer.

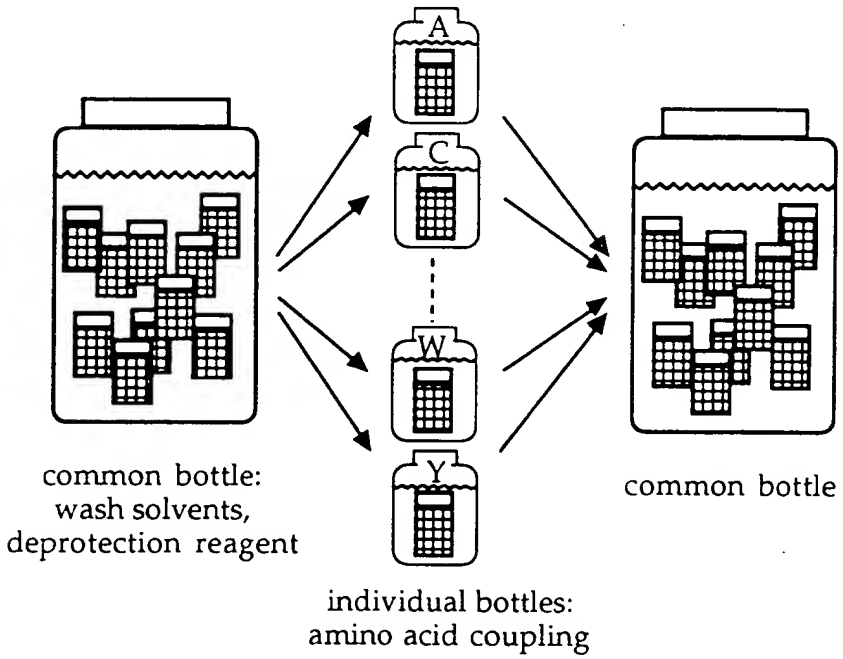

Figure 3. The "tea bag" method of multiple peptide synthesis.

Given that submonomer synthesis allows incorporation of any of over 1,000 readily available primary amines, in principle 10^9 trimers and 10^{12} tetramers can be prepared. This vast combinatorial variety allows a paradigm to select a minimum number of amines to maximize diversity. Toward this objective, flower plots for thousands of amines have been developed; flower plots graphically represent sixteen properties (five finger print multidimensional scaling [MDS] parameters, five atom layer MDS dimensions, five topological index principle components and log P) for a given amine. These flower plots can aid in designing libraries, for example, selecting similar or dissimilar sets of amines for lead discovery and/or lead optimization [Simon et al., 1994]. By combining the sub-monomer synthesis of NSG peptoids using diverse amines, selected with the help of this type of statistical analysis, CHIR 2279 and CHIR 4531 were discovered to have high affinity for the α-adrenergic

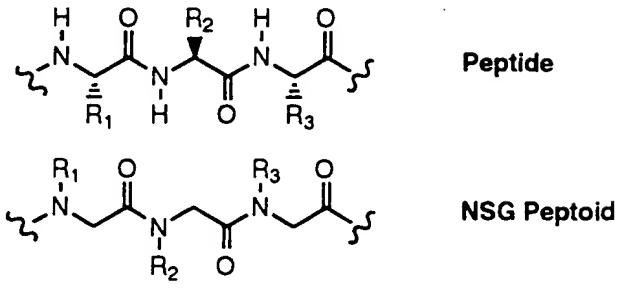

Figure 4. Comparison of a peptide chain and an NSG peptoid chain.

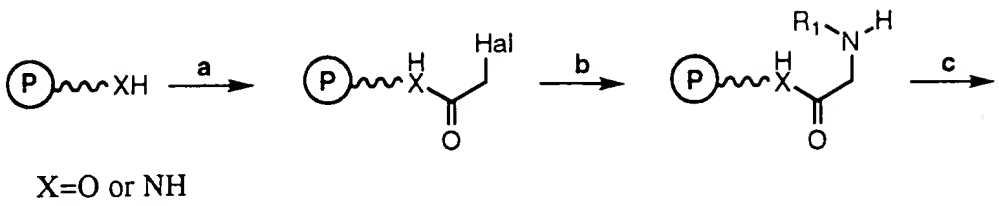

Figure 5. Synthesis of NSG peptoids by the submonomer method. Reagents: (a) DIC, DMF; (b) R-NH_2, DMSO, 25°C; (c) repeat steps a and b.

and μ-opiate receptors, respectively (Fig. 6) [Zuckermann et al., 1994].

"One Bead-One Compound"

Hruby, Lam, and co-workers at Selectide significantly extended SPPS by the application of selectively cleavable linkers [Lebl et al., 1994; Patek and Lebl, 1990, 1991a,b]. The Selectide process is based on: (1) synthesis of diverse compounds on polymeric beads, with a single structure on each particle, (2) screening of this library in either a binding assay based on an enzyme-linked or fluorescent tag or in standard solution-phase assays, and (3) determination of the structure on the "active" bead via sequencing peptides by Edman degradation or using mass spectroscopic techniques [Kocis et al., 1993; Lam et al., 1991, 1993; Lebl et al., 1993; Nikolaiev et al., 1993].

A key feature is that the structure of the peptide on a given bead is not tracked. Rather, it relies on determining the structure only if the synthesized peptide on the bead has biological activity of interest. The library is generated by mixed resin synthesis using 120 μm diameter beads; a single bead with three linkers contains about 300 pmol of peptide. In this approach, a polymeric support is divided into equal portions for coupling to each individual amino acid, and later recombined to create mixtures. Uniform coupling is ensured, since competition between amino acids due to unequal coupling rates is eliminated. Individual oligomers (or polymers) are combined during washing and deprotection, and then separated into individual portions for the next coupling. Resulting peptides exhibit a purely statistical distribution of sequences. Through this combinatorial approach, a complete library of peptides can be prepared rapidly in approximately equimolar amounts.

The assay protocol takes advantage of the selective cleavage of branched linkers. Thus, on each bead three copies of a peptide are synthesized. The scheme (Fig. 7) for assaying peptide libraries involves: (1) dispensing the library at 500 beads/well, releasing ⅓ of each peptide and testing of 500 peptides per well in a biological assay; (2) on finding a particular well having interesting activity, 500 beads are dispensed at one bead per well and activity is deconvoluted to individual peptides using another ⅓ of the peptide; and finally (3) the remaining ⅓ of the peptide on the "active" bead is released and its structure determined by analytical chemistry. Recently, Lebl and co-workers have extended this strategy for the generation of libraries containing amide bonds having non-natural building blocks [Lebl et al., 1994; Salmon et al., 1993].

"Light Directed Chemical Synthesis"

The development of very large scale immobilized polymer synthesis (VLSIPS) resulted from advances in microelectronics, combining solid-phase synthesis with photolithography and miniaturization [Adams et al., 1994; Fodor et al., 1991; Gallop et al., 1994; Pirrung et al., 1992] (Fig. 8). In this technique, the use of photochemically labile protecting groups allows the light-directed, spatially addressable, parallel, chemical synthesis of thousands of oligomers at defined sites on a micromolar scale. Combinatorial masking strategies are used to generate large numbers of compounds in a small number of chemical steps. This technique allows the use of unnatural amino acids, nucleotides, and, in principle, any set of chemically compatible building blocks. The VLSIPS technology has been reviewed [Dower and Fodor, 1991; Gallop et al., 1994; Gordon et al., 1994].

Additional Developments

In addition to these major contributors, Corvas has reported automated synthesis of peptide C-terminal aldehydes as an important class of transition-state analogues (Fig. 9) [Murphy et al., 1992]. This method relies on the protection of an aldehyde as a stereochemically stable semicarbazone. After the desired number of deprotection/coupling cycles are complete, the protected peptide semicarbazone is treated with squeous acid/formaldehyde to regenerate the aldehyde, and then cleaved from the solid support.

A challenge in using combinatorial libraries is

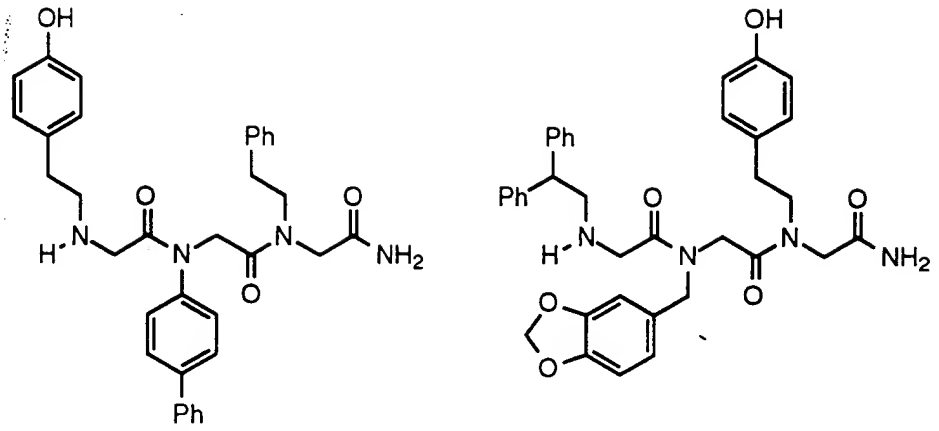

Figure 6. Structures and affinities of NSG peptoids for α1-adrenergic (CHIR 2279) and μ-opiate (CHIR 4531) receptors.

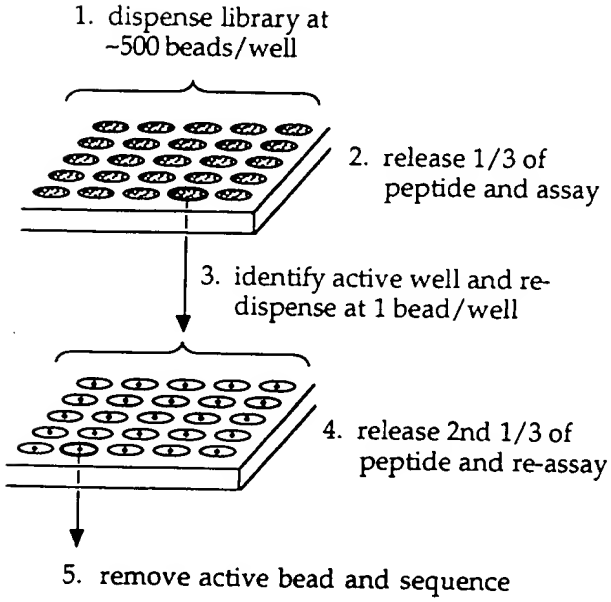

Figure 7. Scheme for discovery of active peptides from a "one peptide per bead" library.

the characterization of members of the library with desired properties. For this, some prefer to rely on iterative resynthesis, while others depend on analytical chemistry. Yet another solution, a "tagging" method, attempts to solve the structure elucidation problem by co-synthesizing a sequenceable tag that encodes a series of steps and reagents used in the synthesis of each library element. The tag and corresponding library element are associated by their attachment to a given resin bead. Once a library element is selected, the procedure used to synthesize it can be read by sequencing the tag. Oligonucleotide [Brenner and Lerner, 1992; Needles et al., 1993] and oligopeptide [Kerr et al., 1993; Nikolaiev et al., 1993] tags have both been reported. Still and co-workers have devised an alternative method that uses a chemically inert, multiple tag labeling scheme, which attaches to the beads tagging molecules that encode both the step number and the chemical reagent used in that step. The array of tags used forms a binary record of the synthetic steps for each bead; tags can be read directly from a single bead by electron capture capillary gas chromatography [Borchardt and Still, 1994; Ohlmeyer et al., 1993].

The convergence of solid-phase synthesis methods with general building block strategies and pharmacophore-driven concepts such as NSG peptoids [Simon et al., 1992], a significant contribution of the last decade, is only now becoming more commonplace. In search of polypeptide-like substances with altered backbones, synthesis of vinylogous polypeptides [Hagihara et al., 1992] and oligocarbamates [Cho et al., 1993; Simon et al., 1991] have been described (Fig. 10). Vinylogous polypeptides consist of repeating units of extended amino acids that have an (*E*)-ethenyl unit inserted between the carbonyl carbon and C_α. The key step in the synthesis of certain oligocarbamates involves the coupling of an amino group with *N*-protected p-nitrophenyl carbonate monomers with a variety of side chains (Fig. 11).

The less explored possibility of generating a library of compounds in solution is very appealing if compounds can be synthesized as mixtures in equal

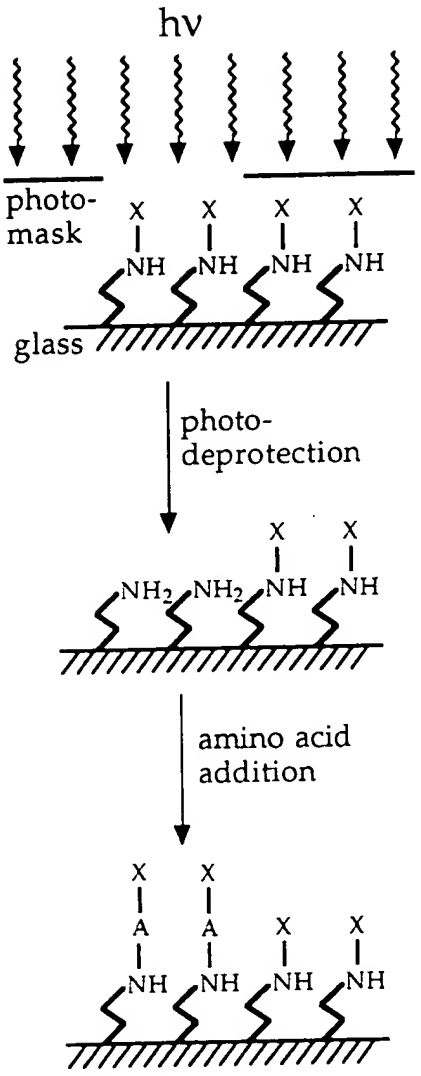

Figure 8. Spatially addressable arrays of peptides can be synthesized on a glass surface using photolabel protecting groups (X).

amounts without side products and byproducts. A recent report of coupling an amine with a carboxylic acid with polymer bound 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (P-EDC) holds considerable promise for this purpose [Desai and Stephens Stramiello, 1993]. The polymer tether enables the urea and N-acyl urea, if any, to remain on the polymer; the product therefore can be isolated simply by filtration and evaporation of the filtrate (Fig. 12).

SYNTHESIS OF NON-AMIDE BASED LIBRARIES

The strategies discussed above produce structures that may have less than optimal bioavailability and pharmacokinetic parameters required for oral delivery of drugs. Many orally available drugs have heterocyclic structures, with MW<500. The development of solid-phase synthesis of heterocyclic compounds is in a most critical stage for the drug discovery process: (1) the combinatorial synthesis of heterocyclic structures on solid phase has the ability to provide a large number of structures for in vitro screening in an assay of biological interest; and (2) the lead structure discovered from these libraries may require less extensive modification to produce suitable drug candidates.

The solid-phase synthesis of non-peptides is not unprecedented, although it is little known to organic chemists. Rapoport [Crowley and Rapoport, 1976] and Leznoff [Leznoff, 1978], are among the early pioneers to explore ambiguous limitations of non-peptide solid-phase chemistry. For example, Dieckmann and other condensation reactions were explored in the 1970s and before [Crowley and Rapoport, 1970, 1976]. The preparation of monoacetates of symmetrical diols [Fyles and Leznoff, 1976] and insect sex attractants [Fyles et al., 1978; Leznoff and Fyles, 1977] has also been accomplished using polymer-supported

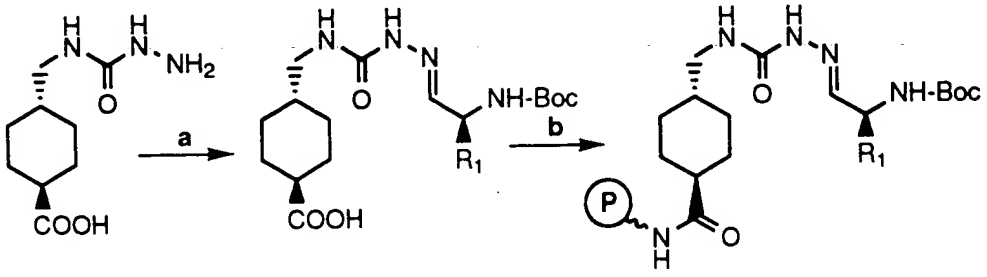

Figure 9. Synthesis of peptide C-terminal aldehydes. Reagents: (a) Boc amino acid aldehyde/NaOAc reflux in ethanol; (b) MBHA resin/(benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluorophosphate.

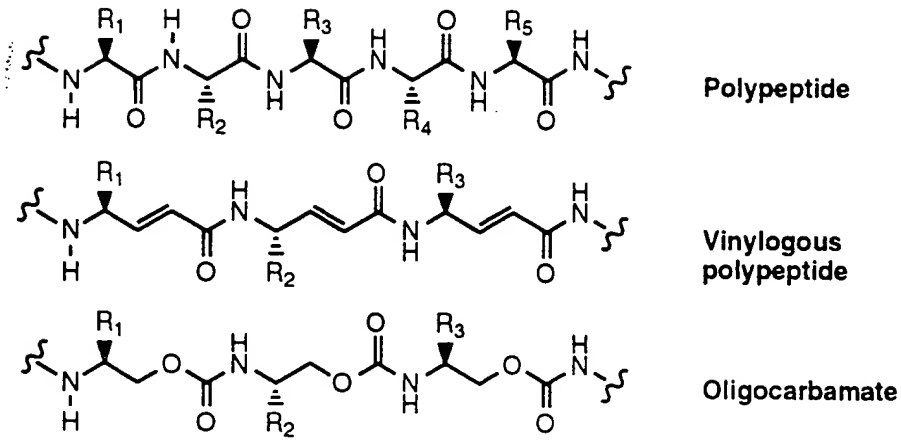

Figure 10. Comparison of portions of peptides, vinylogous polypeptides, and oligocarbamates.

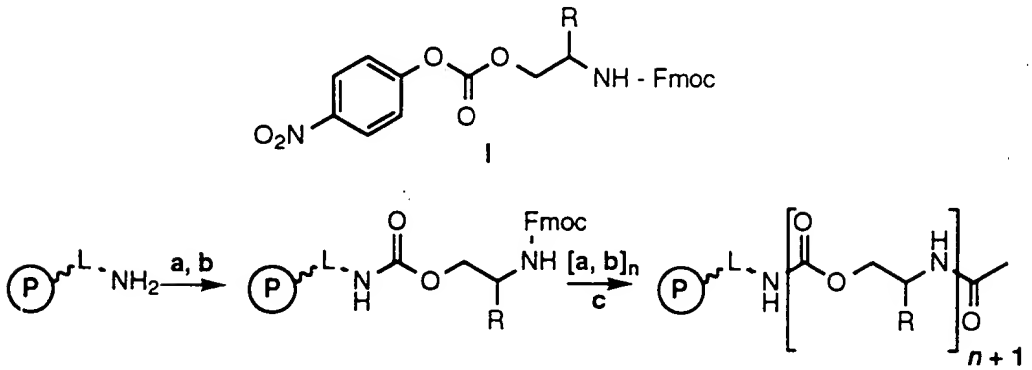

Figure 11. Synthesis of oligocarbamates. Reagents: (a) I, HOBT, diisopropylethylamine, NMP; (b) piperidine, NMP; (c) cleavage with TFA after capping with acetic anhydride.

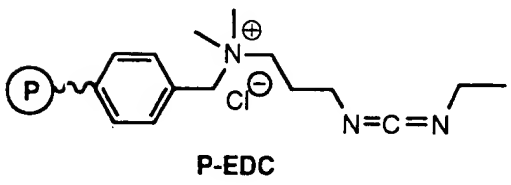

Figure 12. Polymer bound 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (P-EDC).

organic synthesis [Leznoff, 1974, 1978]. The ability to produce compounds with drug characteristics should greatly enhance the drug discovery process. The focus has now been shifted to the synthesis of certain classes of compounds on solid phase and then utilizing the synthetic process for library production. Recent advances in this field are summarized below.

Syntheses f Benzodiazepines and Hydantoins

The ubiquitous 1,4-benzodiazepine nucleus is found in many drugs and ligands with diverse biological activity. Most recently, it has been found in ligands for CCK-A and -B antagonists, tat antagonists, platelet-activating factor antagonists, GPIIb/IIIa inhibitors, reverse transcriptase inhibitors, and ras farnesyltransferase inhibitors [Bunin et al., 1994]. Thus, it is not surprising that 1,4-benzodiazepines were the first class of compounds for which combinatorial synthesis was described. Bunin and Ellman from Berkeley [Bunin and Ellman, 1992; Bunin et al., 1994] and DeWitt et al. from Parke-Davis [DeWitt et al., 1993] independently described the synthesis of benzodiazepine derivatives on polymer support. In Ellman's approach, 1,4-benzodiazepines are constructed on solid support from three components: aminobenzophenones, aminoacids, and alkylating agents (Fig. 13).

The 2-aminobenzophenone derivatives are first attached to the polystyrene solid support employing an acid cleavable linker 4-hydroxymethylphenoxyacetic acid through either an hydroxy or carboxylic acid functionality on one of the aromatic rings of the ami-

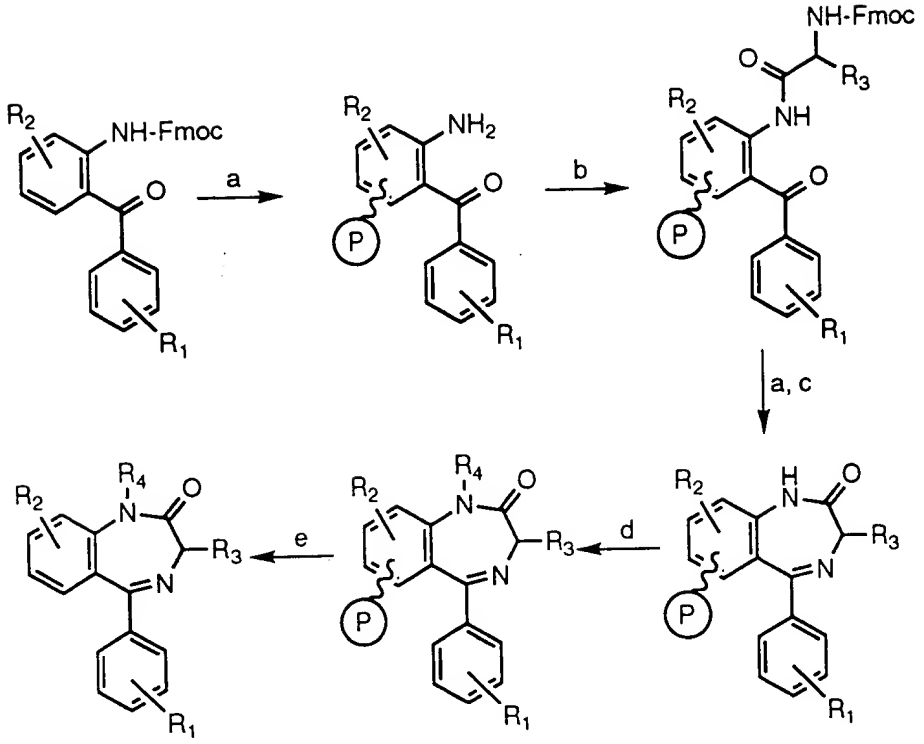

Figure 13. Synthesis of 1,4-benzodiazepine derivatives on solid support. Reagents: (a) 20% piperidine in DMF; (b) N-Fmoc amino acid fluoride, 4-methyl-2,6-di-*t*-butylpyridine; (c) 5% acetic acid in DMF; (d) lithiated 5-phenylmethyl-2-oxazolidone in THF, −78°C, followed by alkylating agent and DMF; (e) TFA/H_2O/Me_2S (85:5:10).

nobenzophenone. Synthesis of the benzodiazepine derivatives on solid support proceeds by removal of the 9-fluorenylmethoxycarbonyl (Fmoc) protecting group from intermediate 2 by treatment with piperidine in DMF; this is followed by coupling the 2-aminobenzophenone to an α-N-Fmoc amino acid fluoride. Deprotection of the Fmoc followed by exposure of the resulting free amine to 5% acetic acid in N-methylpyrrolidone provides the benzodiazepine ring system.

The Parke-Davis strategy relies (Fig. 14) on internal release for formation of the benzodiazepine ring system. The advantage of this strategy is that the compounds that do not "react" remain attached to the resin; this option provides final products in high purity. Through such a synthesis, 2–14 mg of 40 different benzodiazepines corresponding to 9–63% yield of each benzodiazepine were isolated with estimated purities typically >90%.

DeWitt et al. also described the synthesis of hydantoins, a primary nucleus in the anit-epileptic drug phenytoin, and the aldose reductase inhibitor sorbinil [Sarges et al., 1982]. The hydantoin synthesis begins by treating resin bound α-amino acids with isocyanates to provide resin bound ureas. Subsequent cyclization and cleavage provides the hydantoins. This three-step protocol was utilized for the synthesis of 39 hydantoins in 4–81% yield [DeWitt et al., 1993] (Fig. 15).

Nitrile Oxide-Alkene/Alkyne [3+2] Cycloaddition Reactions

Kurth and co-workers reported a multi-step process for the synthesis of 2,5-disubstituted tetrahydrofurans via a tandem 1,3-dipolar cycloaddition/electrophilic cyclization sequence, thereby demonstrating the versatility of polymer supported methodology [Beebe et al., 1992]. The requisite nitro compound for the generation of nitrile oxide was prepared by the Henry reaction of nitromethane with polymer bound aldehyde. After protection of the hydroxyl moiety as a TMS ether, followed by phenylisocyanate mediated dehydration and subsequent 1,3-dipolar cycloaddition with 1,5-hexadiene (2–3-fold excess), this route provided polymer bound isoxazoline (Fig. 16). Finally, electrophilic cyclization of the isoxazoline with iodine monochloride at −78°C gave the 2,5-disubstituted tetrahydrofurans.

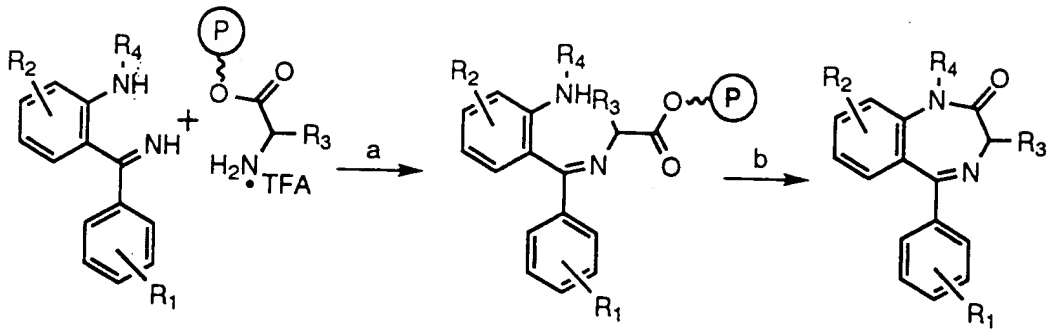

Figure 14. General route for synthesis of benzodiazepines. Reagents: (a) 1,2-dichloroethane, Δ; (b) TFA/Δ.

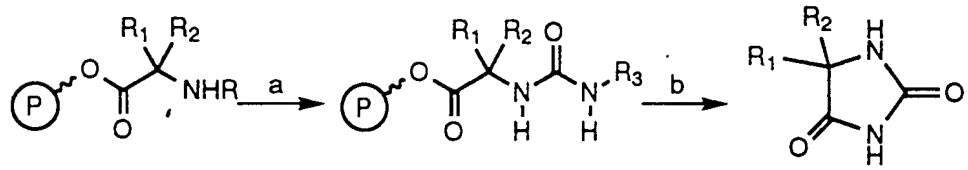

Figure 15. General route for the synthesis of hydantoins. Reagents: (a) R_3NCO, DMF, sonic bath, 6 h; (b) 6M aq. HCl, 85–100°C.

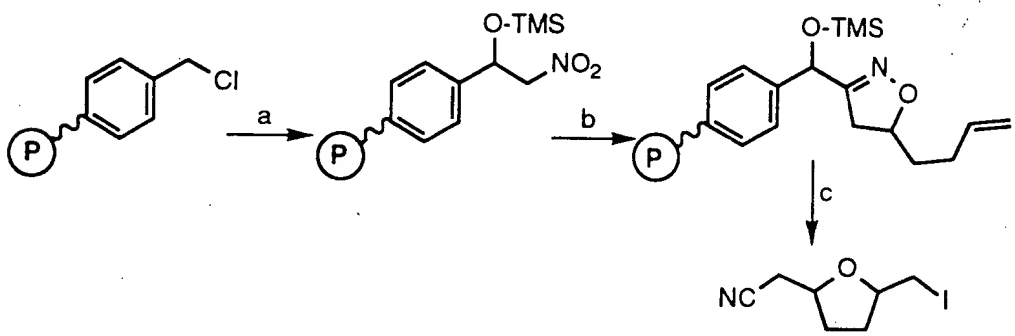

Figure 16. Polymer supported synthesis of 2,5-disubstituted tetrahydrofurans. Reagents: (a) 1. DMSO, $NaHCO_3$, 155°C, 6 h; 2. CH_3NO_2, Et_3N, THF/EtOH, 14 h; 3. TMSCL, Et_3N, THF, 24 h; (b) PhNCO, Et_3N, PhH, 80°C, sealed tube, 1,5-hexadiene, 4 days; (c) ICl, CH_2Cl_2, −78°C, 1.5 h.

Recently, Pei and Moos investigated the formation of isoxazoles and isoxazolines through [3+2] cycloaddition reaction of nitrile oxides with alkynes and alkenes [Pei and Moos, 1994]. In this strategy, NSG peptoids with allyl and alkyne sides chains were synthesized; heating of such NSG peptoids in toluene with nitrile oxides, generated in situ by reacting nitro alkyl compounds with phenyl isocyanate and trimethyamine, afforded the isoxazoles and isoxazolines in >80% purity, respectively (Fig. 17). This "post-modification" of NSG peptoid chains has the potential to create greater diversity than might otherwise possible.

Synthesis of Oligosaccharides Using Polymer-Bound Glycals

Danishefsky and co-workers have re-examined the solid-phase synthesis of oligosaccharides by employing a newly developed stereospecific glycosylation strategy [Danishefsky et al., 1993]. This approach promises to ease some of the drudgery common to the synthesis and purification of oligosaccharides. The technique is applicable to synthesizing glycopeptides and glycosylated proteins, as well as oligosaccharides, and could possibly form the basis for an automated carbohydrate synthesizer. In this approach, glycals

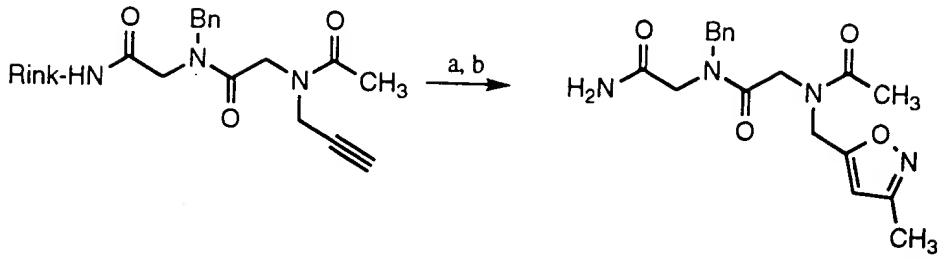

Figure 17. [3+2] Cycloaddition reactions of nitrile oxides on solid phase. Reagents: (a) $C_2H_5NO_2$, PhNCO, TEA, toluene, 100°C; (b) 20% TFA, CH_2Cl_2, room temperature.

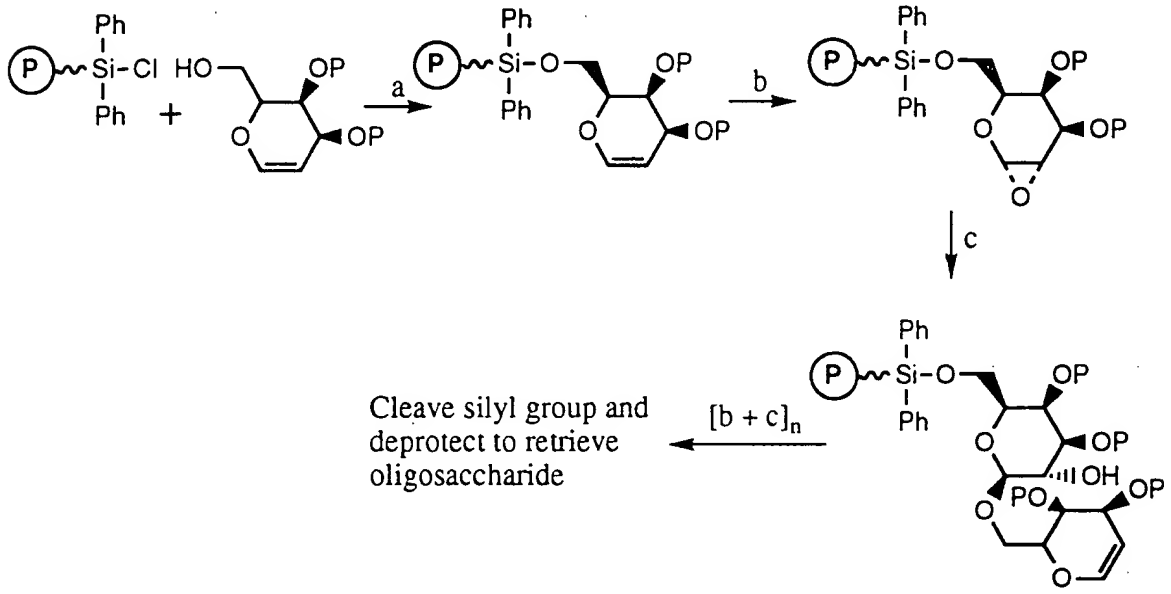

Figure 18. Synthesis of oligosaccharides using polymer-bound glycals. Reagents: (a) Hunig's base and CH_2Cl_2; (b) 3,3-dimethyldioxirane, CH_2Cl_2; (c) I_2, $ZnCl_2$, THF.

are attached to polystyrene co-polymer with a silyl ether bond, and are activated to function as glycosyl donors with 3,3-dimethyldioxirane. Glycosidation is performed by reaction with a solution-based acceptor. Excess acceptor and promoter are removed by rinsing after each coupling, and the desired oligosaccharides are cleaved from the polymer by addition of tetra-n-butylammonium fluoride. The average yield per coupling cycle consisting of epoxidation and glycosidation is ~70% (Fig. 18).

Synthesizing small heterocyclic ring systems that use ligands of diverse biological activity via combinatorial strategies is a fast developing branch of medicinal chemistry. We are at an early state in the development of combinatorial chemistry. However, this dramatic convergence of technologies represents a fundamental advance in medicinal chemistry and promises to play a major role in future drug discovery efforts.

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